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Increased risk of cardiovascular disease may be starting in childhood: 25 OH vitamin D levels in Turkish Children

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Abstract

Background: Vitamin D deficiency has become global health problem and it is associated with metabolic syndrome, type 2 diabetes mellitus, cancer, cardiovascular diseases and increased mortality. The aim of this study is to shed light on the 25 OH vitamin D status in different age groups of Turkish children.

Materials and Method: 1597 children (753 boys (47.2 %) and 844 girls (52.8 %)) admitted to Pediatry Outpatient Clinic were enrolled in this study. 25 OH vitamin D analysis was performed from venous blood samples using Cobas e411 auto-analyzer (Roche Diagnostics, USA).

Results: The children were between 0 and 18 years old, mean of age was 6.90 ± 5.1 years (median: 6 year). 25 OH vitamin D levels were between 3 and 70 ng / mL, mean value was 32.4 ± 16.9 ng / mL (median: 29.1 ng / mL). Age and vitamin D levels were inversely correlated and a statistically significant difference was observed between these two parameters (r=-0.450; p=0.0001). Very low vitamin D levels (<10 ng / mL) differed significantly according to gender. It was found significantly higher in girls (p<0.05).

Conclusion: Vitamin D deficiency is not rare in Turkish children, especially in adolescent period and girls. Vitamin D levels decrease with increasing age throughout childhood. Because of its association with metabolic syndrome, type 2 diabetes mellitus, hypertension and cardiovascular diseases, vitamin D deficiency in childhood may play a role in the development of these diseases in future. Large scale cohort studies are needed to reveal whether there is such an association.

Key Words: Vitamin D, Turkish children, cardiovascular disease, adolescent period

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Introduction

Recent years, it has been revealed that 25-hydroxyvitamin D [25(OH)D] deficiency is associated with metabolic syndrome, type 2 diabetes mellitus, cancer, cardiovascular diseases and increased mortality.(1) It has been also reported that vitamin D deficiency is a global public health problem in all age groups, especially in the Middle East populations.(2) 25(OH)D plays an important role in metabolic regulation such as calcium and phosphate homeostasis, cell differentiation, parathyroid hormone suppression, T-cell suppression, cancer pathogenesis and erythropoiesis.(3, 4) Vitamin D is a fat-soluble vitamin that is naturally present in few foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight strike to skin.

Since vitamin D is biologically inactive, it undergoes two hydroxylations in body for activation. The first occurs in the liver and converts vitamin D to 25-hydroxy vitamin D [25(OH)D, calcidiol]. The second occurs primarily in the kidney and forms physiologically active 1,25-dihydroxy vitamin D [1,25(OH)2D, calcitriol].(5) Serum concentration of 25(OH)D is the best indicator of vitamin D status.(5) It reflects vitamin D produced by skin and obtained from foods and supplements.(5) Vitamin D has a fairly long circulation half-life about 15 days but may change according to racial differences and seasonal changes.(6) Afro-Americans and Hispanics have been reported to have lower vitamin D levels than Caucasians.(7)

Vitamin D deficiency is based on various reasons such as nutrition, atmospheric deterioration, religious clothing, geographical latitude and altitude and seasons affecting daylight. Its deficiency causes rickets in children.(8) Following the observation in 1920 that vitamin D is effective in treating rickets, western countries started to provide this vitamin as a supplement to infants and children and fortified foods such as milk and bread with vitamin D, which led to eradication of vitamin D deficiency as a public health problem in these countries.(9, 10) It is recommended that all infants, children, and adolescents provide a minimum daily intake of 400 IU (10 µg) of vitamin D since the vitamin D status of the newborn is highly related to maternal vitamin D levels.(11) The purposes of this study are to examine 25(OH)D levels and to reveal whether or not there are vitamin D deficiency, differences according to age and gender in Turkish children.

Material and Method

Study population

1597 children [753 boy (47.2%) and 844 girl (52.8%)] admitted to pediatry outpatient clinics of our tertiary training and research hospitals between 2013 and 2014 were enrolled in this study. All of the blood samples were collected during this period. An approval was obtained from the Ethics Committee. 25(OH)D measurements were performed from venous blood samples using Cobas e411 autoanalyzer (*Roche Diagnostics, USA*). ELECSYS® Vitamin D Assay was used to measure serum 25(OH)D levels of the subjects.

Statistical analysis

Statistical analyzes were performed via MedCalc Statistical Software Program (*MedCalc Software version 12.7.7, bvba, Ostend, Belgium; http://www.medcalc.org; 2013*). Descriptive statistical methods (mean, standard deviation, minimum, median and maximum) were used for the definition of continuous variables. For the comparison of more than two independent and normally distributed variables, One-way ANOVA test was used. The comparison of more than two independent and abnormally distributed variables was made with Kruskal Wallis test. For the comparison of two independent and normally distributed variables, Student t test was used.

Mann Whitney U test was used in comparison of two independent and abnormally distributed variables. In the analysis of the associations among categorical variables, Chi Square test and Fisher's Exact test in convenient circumstances were used. P values of <0.05 were considered as significant.

Results

Distributions of participants according to descriptive features, age groups and vitamin D levels have been shown in **Table 1**. A total of 1597 participants aged between 0 and 18 years were enrolled in the analysis. Participants were divided three age groups as 0-2, 3-11, 12-18 years. They also divided four groups according to their vitamin D results as ≤ 10, 11-29, 30-39 and ≥ 40 ng / mL. Vitamin D levels were measured between 3 to 70 ng / mL, mean 32.4 ± 16.9 ng / mL and median

29.04 ng / mL. 844 (52.8%) of 1597 participants were female and 753 (47.2%) were male. The average age of the participants is 6.9 ± 5.1 years.

According to age groups, there is a statistically significant difference in 25OH vitamin D distribution ($p<0.05$). Distributions of Vitamin D levels according to age and gender are shown in **Table 2** and **Table 3**. According to post-hoc binary comparison results; there is a statistically significant difference in terms of distribution of 25OH Vitamin D according to all age groups (Mann-Whitney U test, Bonferroni correction, $p<0.016$, **Table 3**). According to sex, it was determined that there was a significant difference between the rates of the cases in the vitamin D groups (Chi-Square $p<0.05$). It was determined that 65.2% of the cases with vitamin D level ≤10 ng / mL were female.

In the other vitamin D groups, it was determined that the ratios are close to each other. It can be said that this significant difference according to sex is the ones with vitamin D level ≤10 ng / mL. This significant difference has no significant effect on the cumulative (**Table 2**, $p>0.05$). There was a significant relationship between age and vitamin D levels in this study. There is a statistically significant difference in terms of 25OH

Table 1. Distributions of participants according to descriptive features, age groups and vitamin D levels

		Min - Max (Median)	Mean ± SD
Age (years)		0-18 (6)	6.9 ± 5.1
25OH Vitamin D (ng / mL)		3-70 (29.04)	32.4 ± 16.9
		n	%
Gender	Girls	844	52.8
	Boys	753	47.2
Age (years)	0-2	471	29.5
	3-11	753	47.2
	12-18	373	23.4
25OH Vitamin D (ng / mL)	≤10	115	7.2
	11-29	705	44.3
	30-39	301	18.9
	≥40	471	29.6

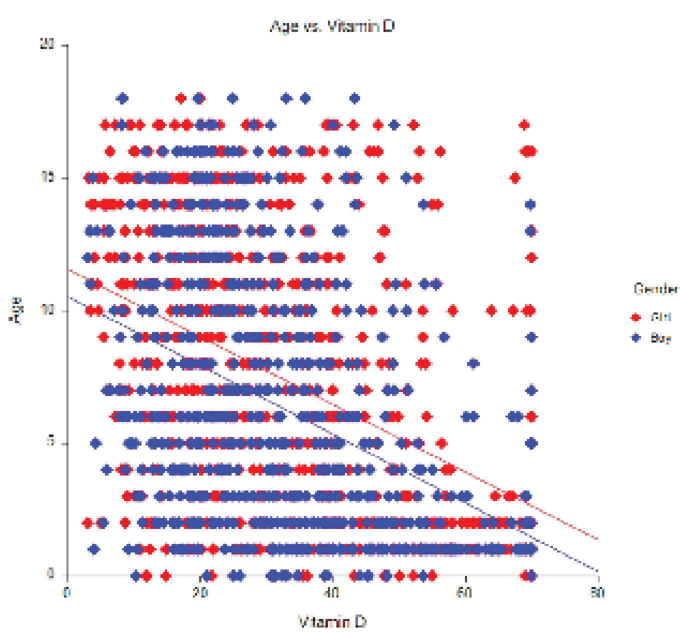
vitamin D groups according to age groups ($p<0.05$). 91.3% of cases with vitamin D levels of 10 or less were in the 3-18 age group, 54.6% of the cases with vitamin D level 11-29 were in the 3-11 age group, 56.1% of cases with vitamin D level 30-39 were in the 3-11 age group, and 59.42% of the cases with vitamin D level 40 and above were in the 0-2 age group.

There was a statistically significant (r:-0.452, p=0.0001) negative relationship between vitamin D levels and age of all children participating in the study. Vitamin D levels decrease with increasing age. This negative relationship was seen both girls (r: -0.434, p=0.001) and boys (r: -0.463, p=0.001, **Table 4** and **Figure 1**).

Discussion

This study demonstrated that age and vitamin D levels were inversely correlated. Vitamin D levels decrease with increasing age in Turkish children (r=-0.450; p=0.0001). Very low vitamin D levels (≤10 ng / mL) also known as vitamin D deficiency were found significantly higher in girls than boys ($p<0.05$). The best and only natural source of vitamin D is sunlight. Unlike dietary or supplementary vitamin D, when one get vitamin D from sunshine, body takes what it needs, and metabolizes. The care of the physician and mother

Figure 1. Graphical representation of inverse relationship between vitamin D and Age



may protect the children until preadolescence period from vitamin D deficiency, but the low levels of vitamin D in children older than 10 years may be due to dietary behavior characteristics and sedentary lifestyle.

In consistent with this, our findings show that vitamin D levels appear to decrease with increasing age from zero to eighteen. The normal level of serum vitamin D in children and adolescents as well as in adults is still a topic of debate. Studies have shown that over half of infants, children, and adolescents are inadequately supplemented. In 2008, the American Academy of Pediatrics published a review article with recommended target vitamin D concentrations for healthy infants, children, and adolescents.[12] Vitamin D has important roles in body metabolism. Parathormon (PTH) levels rises in low vitamin D concentrations, and increases blood pressure, and exerts several negative effects on heart such as myocardial hypertrophy and arrhythmias.[5]

Suppression of PTH via vitamin D supplementation reduces cardiovascular risk and high blood pressure.[5] Vitamin D suppresses renin transcription and reduces renin angiotensin aldosterone system activity, and has also anti-diabetic, anti-inflammatory and anti-auto immunological properties.[5] The immunologic effects of vitamin D have stimulated great interest, but studies in these areas are currently limited in pediatric patients.[12] Vitamin D has been shown to suppress pro-inflammatory cytokines and promote anti-inflammatory ones in children and adults with congestive heart failure.[13] In murine cardio-myocytes, vitamin D appears to have rapid effects on contractility and relaxation, which improve cardiovascular function.[15]

Moreover, vitamin D up regulates insulin receptors and reduces insulin resistance and development of diabetes mellitus type 1.[5] Vitamin D has anti-infectious and anti-inflammatory actions; it reduces inflammation marker tumor necrosis factor-α and increases anti-in-

Table 2. Score comparisons of vitamin D levels with age and gender

		25OH Vitamin D (ng/mL)	
		Mean ± SD (Med.) Min-Max	p
Gender	girls	31.79 + 17.2 28.52 (3-70)	0.062[1]
	boys	33.16 + 16.6 30.59 (3-70)	
Age (years)	0-2	44.48 + 17.3 45.2 (3-70)	<0.001[2]
	3-11	29.13 + 13.8 27.07 (3.41-70)	
	12-18	23.82 + 13.5 21.46 (3-69.99)	
Post Hoc binary comparisons		0-2 vs. 3-11	<0.001[1]
		0-2 vs. 12-18	<0.001[1]
		3-11 vs. 12-18	<0.001[1]

[1]Mann Whitney U test, [2]Kruskal Wallis test

Table 3. Categorical comparison of vitamin D levels with age and gender

		25OH Vitamin D (ng/mL)				p
		≤10	11-29	30-39	≥40	
Gender	Girls (n, %)	75 (65.2)	381 (54.0)	146 (48.5)	241 (51.2)	0.016[1]
	Boys (n, %)	40 (34,8)	324 (46,0)	155 (51,5)	230 (48,8)	
Age (years)	0-2 (n, %)	10 (8.7)	92 (13.0)	89 (29.6)	280 (59.4)	
	3-11 (n, %)	49 (42.6)	385 (54.6)	169 (56.1)	146 (31.0)	<0.001[1]
	12-18 (n, %)	56 (48.7)	228 (32.3)	43 (14.3)	45 (9.6)	

[1]Chi square test

flammatory cytokine interleukin-10.[5] Vitamin D has also anti-hypertrophic and anti-proliferative effects; it reduces matrix metalloproteinase-9 levels.[5] Vitamin D possesses protective effects against atherosclerosis, vascular calcification and endothelial dysfunction; it inhibits macrophage cholesterol uptake and foam cell formation, vascular smooth muscle cell proliferation and migration, and suppresses inflammation triggered endothelial activation and expression of endothelial adhesion molecules.[5]

Several epidemiologic and clinical studies take an attention the association between low vitamin D levels and increased risk of metabolic syndrome, obesity, diabetes mellitus, hypertension and cardiovascular disease risk factors.[16, 17] Pacifico et showed that low levels of vitamin D has associated with metabolic syndrome components such as insulin resistance, central obesity, hypertension, low high density lipoprotein and high triglyceride levels in children and adolescents, but they found that no correlation between vitamin D and impaired flow mediated vasodilatation and increased carotid intima- media thickness which are two markers of subclinical atherosclerosis.[17]

On the other hand, Juonala et al, have reported that there is an association between low childhood vitamin D levels and increased carotid intima-media thickness in adulthood after thirty years observation.[18] Epidemiological studies showed low vitamin D levels in patients with cardiovascular and cerebrovascular diseases, and significant associations between low vitamin D levels and risk of fatal cardiovascular and cerebrovascular events such as sudden cardiac death and fatal strokes.[5] It has also been reported that, beyond cardiovascular disease, there is an association between vitamin D deficiency and all-cause mortality.[5] All of these reports prompted our interest a potential role of vitamin D deficiency on future cardiovascular disease in Turkish children. Similar to findings reported from Europe,[19] we exposed that a large proportion of adolescents in our country has low vitamin D levels. We have also demonstrated that very low vitamin D levels were higher in girls compared to boys, therefore possibly contributing to their lower peak bone mass and also may be increasing trend to CHD.

This difference may be explained by gender differences in body size, muscle mass, as well as possibly in bone remodeling during a critical period for bone mass increment, that is, puberty. And also, in Turkey, nearly half of girls of adolescent age wear sun-resistant clothing and spend most of their times in closed places. Hag et al reported similar result from Abu Dhabi, United Arab Emirates.[20] Science et al have also reported low levels of vitamin D levels in Canadian adolescents.[21] All children, particularly girls, should spend more time outside for the vitamin D synthesis. In summary, our findings showed that even in sunny environments, vitamin D deficiency is quite common. Although Turkey is a Mediterranean country, Turkish people should have checked their levels of 25 (OH)D at periodic intervals throughout their lives including childhood period. Furthermore, proper supplementation should be considered to avoid a secondary serious disorders such as diabetes mellitus, hypertension and coronary artery disease that may arise from 25-(OH)D deficiency.

On the other hand, there are some limitations in this study. Firstly, the seasonal changes of vitamin D levels were ignored. Secondly, we had no knowledge of the paticipants's body mass index, degree of sun exposure. A high body mass index is proportional to lower 25(OH)D values, and this is possibly due to the lipid-soluble vitamin D stored in fat tissues, which causes a reduction in serum levels.[22, 23] Given the important role

Table 4. Assessment of the relationship between Vitamin D levels and age of children

		Vitamin D vs Age		
		Total	Girls	Boys
All participants (n=1592)	n	1592	843	749
	r	-0.450	-0.434	-0.463
	p	0.0001**	0.001	0.001**

r: *Spearman's correlation co-efficient* of vitamin D in childhood health, more time spent in outdoor activity for sunlight exposure and vitamin D supplementation may be necessary for optimal health in infants, children, and adolescents. And also, optimal vitamin D levels in the mother during pregnancy should be maintained. Moreover, with the onset of puberty, there is acceleration in rate of growth and bone mass, which leads to an increase in requirements for vitamin D and calcium.[24] A program consisting of measurement and replacement of vitamin D to adolescents in healthcare system may be initiated.

Conclusion

Vitamin D deficiency is not rare in Turkish children, especially in adolescent period and girls. Vitamin D levels decrease with increasing age throughout childhood. Because of its association with metabolic syndrome, hypertension, type 2 diabetes mellitus and cardiovascular diseases, vitamin D deficiency in childhood may play a role in development of these hazardous diseases in future. Large scale cohort studies are needed to reveal whether there is such an association.

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